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# Studies on doses of methimazole (MMI) and its administration regimen on broiler metabolism ☆

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## Abstract

We designed three experiments to determine both the optimal dose of and time on experiment for methimazole (MMI; 1-methyl-2-mercaptimidazole). Our goals were to determine if chicken growth was related to thyroid hormone levels and if intermediary metabolism changed along with changes in thyroid hormone levels. Initiating MMI at one week of age decreased ($P < 0.01$) plasma thyroid levels and growth in four-week old birds. In contrast, initiating MMI at two and three weeks of age decreased ($P < 0.05$) hormone levels without affecting growth as severely. Although initiating MMI at two weeks of age depressed ($P < 0.05$) plasma thyroid hormones at four weeks, there was little change in vitro lipogenesis at four weeks. Again, initiating MMI at one week of age decreased body weight, plasma thyroid hormones and in vitro lipogenesis at four weeks of age. In addition, this treatment also decreased ($P < 0.05$) malic enzyme activity at this same age period. The second experiment showed that MMI, initiated at 14 days, had no significant effect on 28-day body weight and again decreased both plasma $T_3$ and $T_4$ but $T_3$ replacement increased plasma $T_3$ in both 14–28-day treatment groups. All body weights were similar at 30 days, however. Lastly, diets containing graded levels of MMI decreased thyroid hormones and body weight ($0 > 0.25 > 0.5 > 1$ g MMI/kg). In contrast, only the two higher levels (0.5 and 1 g MMI/kg) decreased in vitro lipogenesis. Growth depression, caused by MMI feeding, can occur without changes in lipid metabolism. The length of MMI administration may be as important as dose level in obtaining effects (growth, thyroid hormone depression and inhibition of lipogenesis).
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## 1. Introduction

Although the thyroid gland partially controls avian growth, artificial changes in thyroid hormone levels do not always change growth predictably. In one study (Leung et al., 1985), dietary triiodothyronine ($T_3$) and thyroxine ($T_4$) decreased body weight and feed efficiency of chickens. In a previous study (Leung et al., 1984a,b), daily injections of thyroid releasing hormone (TRH) improved growth and increased plasma thyroid hormone concentrations. In contrast, feeding $T_3$ increased plasma $T_3$, but failed to improve the growth weight of dwarf chickens (Leung et al., 1984a,b). Cogburn et al. (1989) also reported that dietary TRH increased plasma growth hormone (GH), thyroid hormone levels and body weight. On the other hand, long-term, dietary administration of thyroid hormones in another study decreased both growth and fat deposition, with $T_3$ being more effective than $T_4$ (Decuypere et al., 1987). Other sets of data also suggested that dietary $T_3$ decreased body fat (Leung et al., 1984a,b) as well as plasma GH concentrations (Harvey, 1983). It should also be noted that chemical hypothyroidism, caused by either propylthiouracil (PTU) or methimazole, also decreased growth (Leung et al., 1985; Chiasson et al., 1979).

What is lacking from these reports is any information concerning birds' recovery from thyroid hormone perturbations (inhibition of $T_3$ production). We proposed and tested the hypothesis that feeding $T_3$ after a three-week methimazole

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challenge would rapidly restore circulating levels of $T_3$. Methimazole (1-methyl-2-mercaptimidazole), used to induce hypothyroid status in the present experiments, inhibits thyroidal production of thyroid hormones, but does not directly affect extrathyroidal $5'$ deiodination of $T_4$. Previous studies lacked rigorous examinations of both MMI doses and time required for the effects of hypothyroidism. It was of interest to determine if perturbations in thyroid metabolism could change indices of intermediary metabolism without changes in body weight.

Lipogenesis was noted by monitoring the incorporation of a substrate into hepatic fatty acids in vitro. This measurement can be used to approximate the in vivo state, also. Malic (ME) enzyme activity was monitored because of its role in providing reducing equivalents (NADPH) for the synthesis of fatty acids. Isocitrate : NADP$^+$ oxidoreductase-[decarboxylating] (ICD) may function as both a residual source for the provision of NADPH and to provide a coreactant for transamination. Aspartate aminotransferase (AAT) aids in the removal of excess amine groups formed by feeding high-protein diets (Rosebrough et al., 1988).

## 2. Materials and methods

### 2.1. Animals and diets

Male Hubbard $\times$ Hubbard broiler chickens (*Gallus gallus*) were held under a quarantine that was certified by the station veterinarian and observed daily for healthiness. One authorized animal caretaker was assigned to maintain chickens over the course of the experiments. In addition, the research protocols were approved by the Beltsville Agricultural Research Animal Care Committee. Birds were allowed to consume both feed and water on an ad libitum basis. Birds were kept in electrically heated battery-brooders (4 birds/pen replicate) in an environmentally controlled room (22 °C). A 12-h light (0600 to 1800 h), 12-h dark (1800 to 0600 h) cycle was maintained. The birds were killed by decapitation at 0900 h to minimize possible diurnal variation.

In Experiment 1, we used a dose of MMI (1 g/kg diet) known to produce both changes in plasma thyroid hormones and intermediary metabolism (Rosebrough and McMurtry, 2003). Our usual approach has been to initiate this dietary treatment in 7-day old birds and continue for 21 days, sampling at 28 days. Historically, this regimen depresses both feed intake and body weight, possibly confounding changes in intermediary metabolism. In this experiment, we modified our usual protocol by initiating MMI at 14 and 21 days in addition to the usual initiation at one week. Blood samples were taken at two, three and four weeks of age to determine relationships among plasma thyroid concentrations, dosing intervals and final body weights (four weeks of age).

A total of 144 chickens (two replications of 72 chickens each) were used in Experiment 2 which was conducted as a follow up to the first experiment. Briefly, it was noted that initiating methimazole at two weeks of age gave a body weight at four weeks that equaled the untreated controls yet decreased plasma thyroid hormone concentrations. Birds were fed the diet containing the same quantity of MMI, but the treatment was initiated when the birds were two weeks of age. Birds were selected and sampled at four weeks of age. In addition, birds from these treatments were also given supplemental $T_3$ from 28 to 36 days (Rosebrough and McMurtry, 2003) to determine if this two to four week dosing regimen would affect responses in intermediary metabolism during this period and at seven weeks of age (all birds on a common diet for two weeks).

A total of 48 chickens were used in Experiment 3 to test an MMI dose response regimen. Birds were fed diets containing 0, 0.25, 0.50 and 1 g of MMI/kg diet. These diets were initiated when birds were one week of age and continued until four weeks of age.

### 2.2. In vitro metabolism — lipogenesis

Birds were then selected (day 28) and killed by decapitation at 0900 h to minimize possible diurnal variation. Livers were rapidly excised into phosphate buffered saline, rinsed to remove debris and held at 2 °C for later use for in vitro lipogenic studies. Livers were then sliced (MacIlwain Tissue Chopper; 0.4–0.5 mm) and quadruplicate explants were incubated at 37 °C for 2 h in Hanks' balanced salts (Hanks and Wallace, 1949) containing, 10 mM-HEPES and 10 mM-sodium[2-$^{14}$C]acetate (166 MBq/mol). All incubations were conducted in 3-ml volumes at 37 °C for 2 h under a 95% $O_2$– 5% $CO_2$ atmosphere (Rosebrough et al., 1988; Rosebrough and Steele, 1985, 1987). At the end of the stated incubation periods, the explants were placed in 10 mL of 2 : 1 chloroform : methanol for 18 h according to Folch et al. (1957). The extracts were evaporated to dryness and dispersed in scintillation fluid. Radioactivity in the extracts was measured by liquid scintillation spectroscopy. In vitro lipogenesis was expressed as micromoles of acetate incorporated into lipids per gram of tissue.

### 2.3. In vitro metabolism — enzyme assays

Remaining liver tissues were homogenized (1 : 10, wt/vol.) in 100 mM-HEPES (pH 7.5) 3.3 mM-mercaptoethanol and centrifuged at 12,000 $\times g$ for 30 min (Rosebrough and Steele, 1985). The supernatant fractions were kept at $-80$ °C until analyzed for the activities of malate : NADP$^+$ oxidoreductase-[decarboxylating] (ME, EC 1.1.1.40), isocitrate : NADP$^+$ oxidoreductase-[decarboxylating] (ICD-NADP, EC 1.1.1.42) and aspartate aminotransferase (AAT, EC 2.6.1.1). The activity of ME was monitored because of the enzyme's role in providing reducing equivalents (NADPH) for the synthesis of fatty acids. Isocitrate : NADP$^+$ oxidoreductase-[decarboxylating] may function as both a residual source of NADPH and to provide a coreactant for transamination.

Malic enzyme activity was determined by a modification of the method of Hsu and Lardy (1969). Reactions contained 50 mM-HEPES (pH 7.5), 1 mM NADP, 10 mM $MgCl_2$ and the substrate, 2.2 mM L-malate (disodium salt) in a total volume of 1 mL. Portions (50 μL) of the 12,000 $\times g$ supernatants

(diluted 1:10) were preincubated in the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 30 °C.

Isocitrate:NADP$^+$ oxidoreductase-[decarboxylating] activity was determined by a modification of the method of Cleland et al. (1969). Reactions contained 50 mM HEPES (pH 7.5), 1 mM NADP, 10 mM MgCl$_2$ and the substrate, 4.4 mM DL-isocitrate (substrate) in a total volume of 1 mL. Portions (50 µL) of the 12,000 $\times g$ supernatants (diluted 1:10) were preincubated in the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 30 °C.

Aspartate aminotransferase activity was determined by a modification of the method of Martin and Herbein (1976). Reactions contained 50 mM-HEPES, 200 mM-L-aspartate, 0.2 mM-NADH, 1000 U/L malate:NAD$^+$ oxidoreductase (EC 1.1.1.37) and the substrate, 15 mM-2-oxoglutarate in a total volume of 1 mL. Portions (25 µL) of the 12,000 $\times g$ supernatants (diluted 1:20) were preincubated in the presence of the first four ingredients. Reactions were initiated by adding the substrate and following the rate of oxidation of NADH at 340 nm at 30 °C. Enzyme activities are expressed as micromoles of product formed per minute under the assay conditions (Rosebrough and Steele, 1985, 1987).

### 2.4. Plasma thyroid hormone concentrations

For Experiments 1 and 2, T$_3$ and T$_4$ concentrations were estimated with commercially available solid-phase single antibody radioimmunassay (RIA) kits (ImmuChem™ Triiodothyronine and ImmuChem™ Thyroxine, ICN Biomedicals, Irvine, CA, USA). These assays were validated for avian samples (Rosebrough et al., 1988) by dispersing standards in charcoal-stripped chicken serums and by noting recovery of added T$_3$ and T$_4$ (98%). For Experiment 3, T$_3$ and T$_4$ concentrations were estimated with a double antibody RIA (Newcombe et al., 1992). The latter procedures became necessary because the vendor no longer provided solid phase, polyclonal antisera in the solid phase kits. The currently provided monoclonal antibody in the T$_4$ kits did not prove acceptable for avian samples.

### 2.5. Plasma metabolites

Plasma triglycerides, uric acid and nonesterified free fatty acids were determined with commercially available kits (Sigma Chemical Bulletin Nos. 334-UV and 292-UV, Sigma Chemical Co., St. Louis, MO, USA; NEFA-C, Wako Pure Chemical Industries LTD, Osaka, Japan).

Plasma glucose and lactate were measured with a YSI Biochemistry Analyzer (2700) equipped with a 2365 dextrose membrane (procedure 311; glucose) or a 2329 membrane (procedure 301; lactate). Both procedures measure hydrogen peroxide amperometrically and current flow is proportional to the rates of substrate (metabolite) initially present. Each

hormone or metabolite was measured in a single assay to remove inter-assay variation.

### 2.6. Statistical analyses

Data were analyzed according to various models presented in Remington and Schork (1970). Comparisons of individual means were accomplished by using pooled standard errors arising from analyses of variance.

## 3. Results

Table 1 (Experiment 1) summarizes effects of MMI administration on plasma thyroid hormone concentrations at three age periods as well as 28-day body weights. The data reveal a significant ($P<0.05$) dose response relationship between plasma T$_3$ at 28 days and the time of initiating the dietary MMI. Initiating MMI at seven days of age decreased ($P<0.05$) plasma T$_3$ and body weight at 28 days compared to the other treatments. In contrast, initiating MMI at 14 days also decreased plasma T$_3$ at 28 days, but, in contrast, did not decrease 28-day body weight.

Experiment 2 took the above response concerning the initiation MMI at 14 days and expanded the protocol to study repletion of plasma thyroid hormone levels with exogenous T$_3$. Tables 2–4 summarize the 8-day effects (28–36 days) of T$_3$ replenishment and subsequent effects while Table 5 summarizes these treatments after feeding a common control diet for an additional 13 days (36–49 days). As in Experiment 1, MMI, initiated at 14 days, had no significant effect on 28-day body weight but, again, decreased both plasma T$_3$ and T$_4$ ($P<0.05$; Table 2). Day 30 was the first measured increment of T$_3$ replacement. All body weights were similar at 30 days although, as expected supplemental T$_3$ did increase plasma T$_3$ ($P<0.05$) in both 14–28-day treatment groups. In contrast, plasma T$_4$ was significantly greater ($P<0.05$) in controls when compared to the other groups. Supplemental T$_3$ increased ($P<0.05$) plasma T$_3$, regardless of the 14–28-day treatment.

Table 1
Effects of time of initiation of methimazole (MMI) administration on responses of broiler chickens (growth and plasma thyroid hormones)[1]

| Age | Treatment | T$_3$, ng/ml | T$_4$, ng/ml | Weight at 28 days |
|---|---|---|---|---|
| 7 days | Basal | 3.1±0.1 | 11.3±1.1 | |
| 14 days | Control | 3.0±0.2[b] | 9.3±0.9[b] | |
| | MMI from 7 days | 1.1±0.1[a] | 3.9±0.1[a] | |
| 21 days | Control | 2.7±0.1[b] | 8.9±1.6[b] | |
| | MMI from 7 days | 1.8±0.4[a] | 4.6±0.7[a] | |
| | MMI from 14 days | 2.2±0.6[ab] | 3.7±0.2[a] | |
| 28 days | Control | 2.3±0.2[d] | 11.5±1.3[b] | 1051±68.9[b] |
| | MMI from 7 days | 0.8±0.1[a] | 4.2±0.3[a] | 731±110.5[a] |
| | MMI from 14 days | 1.2±0.1[b] | 4.3±0.3[a] | 1025±100.3[b] |
| | MMI from 21 days | 1.6±0.1[c] | 3.4±0.3[a] | 1051±32.3[b] |

[a,b,c,d] Values within an age period with common superscripts are similar ($P<0.05$).
[1] Birds were fed diets containing 1 or 0 g of MMI per kilogram starting at 7, 14 or 21 days of age. Plasma thyroid hormones were measured at the stated intervals and body weights at 28 days.

Table 2
Effects initiating methimazole (MMI) treatment at 14 days on responses of broilers given supplemental $T_3$ on growth and plasma thyroid hormones[1] (Experiment 2)

| 28 day diets | Age | 28–36 day diets | Weight | $T_3$ | $T_4$ |
|---|---|---|---|---|---|
| | | | g | ng/ml | |
| Control | 28 days | | $1134\pm52.6^a$ | $2.5\pm0.1^b$ | $10.3\pm0.5^b$ |
| MMI | 28 days | | $1048\pm67.3^a$ | $1.2\pm0.1^a$ | $3.8\pm0.3^a$ |
| | | | | | |
| Control | 30 days | Control | $1349\pm87.2^a$ | $2.6\pm0.2^a$ | $12.6\pm2.8^b$ |
| Control | 30 days | $+T_3$ | $1214\pm49.3^a$ | $20.1\pm0.9^b$ | $5.0\pm0.1^a$ |
| MMI | 30 days | Control | $1149\pm68.0^a$ | $3.8\pm0.4^a$ | $6.1\pm0.1^a$ |
| MMI | 30 days | $+T_3$ | $1105\pm41.1^a$ | $16.1\pm2.9^b$ | $4.6\pm0.5^a$ |
| | | | | | |
| Control | 33 days | Control | $1566\pm58.2^c$ | $2.9\pm0.1^a$ | $8.6\pm1.2^b$ |
| Control | 33 days | $+T_3$ | $1393\pm46.3^b$ | $7.2\pm0.9^b$ | $3.3\pm0.4^a$ |
| MMI | 33 days | Control | $1281\pm32.6^{ab}$ | $3.1\pm0.1^a$ | $7.0\pm0.2^b$ |
| MMI | 33 days | $+T_3$ | $1185\pm82.4^a$ | $7.3\pm1.7^b$ | $2.7\pm0.5^a$ |
| | | | | | |
| Control | 36 days | Control | $1768\pm29.5^b$ | $2.5\pm0.4^a$ | $13.1\pm1.2^c$ |
| Control | 36 days | $+T_3$ | $1523\pm80.1^a$ | $10.9\pm0.3^c$ | $5.3\pm0.1^b$ |
| MMI | 36 days | Control | $1415\pm42.3^a$ | $2.6\pm0.1^a$ | $7.3\pm0.2^b$ |
| MMI | 36 days | $+T_3$ | $1425\pm67.4^a$ | $8.1\pm2.1^b$ | $3.5\pm.0.5^a$ |

[a,b,c,d] Values within an age period with common superscripts are similar ($P<0.05$).
[1] Birds were fed diets containing 1 or 0 g of MMI per kilogram from 14 to 28 days of age. One-half of the birds were then fed a diet containing 1 mg of $T_3$ from 28 to 36 days of age. The other half of the birds were fed an unsupplemented diet.

Trends noted at 36 days were similar statistically to those noted at 33 days.

Table 3 summarizes both the 14–28-day effects of MMI and $T_3$ replenishment on some indices of intermediary metabolism. Methimazole decreased ($P<0.05$) ME activity at 28 days but did not affect IVL, ICD or AAT activities. In vitro lipogenesis at 30 days was the same for birds given the control diet for both experimental periods and for those given MMI for the first period (14–28 days) and the control diet for the second period (28–36 days). In vitro lipogenesis was least ($P<0.05$) in birds given the control diet for the first period and $T_3$ for the second period and greatest ($P<0.05$) for birds given MMI for the first period but $T_3$ for the second period. In contrast, sequential observations at both 33 and 36 days revealed equal rates of IVL in birds fed the control diet during the second period, regardless of first period diets. Likewise, in stark contrast to the findings at 30 days, supplemental $T_3$ decreased IVL ($P<0.05$) in birds fed MMI during the first period. Of particular interest, was the apparent stimulation of ME activity ($P<0.05$) at day 30 in birds given supplemental $T_3$ after being fed MMI during the first period. Birds fed MMI in the first period and the control diet during the second period showed a gradual increase in ME that appeared to lag behind the MMI birds given $T_3$. Both groups given MMI in the first period had similar ME activities at days 33 and 36, thus discounting any further stimulation by supplemental $T_3$. Moreover, supplemental $T_3$ actually depressed ME activity at 33 and 36 days.

Neither of the first period diets nor supplemental $T_3$ in the second period changed plasma glucose during the time increments of the second period (Table 4). Although there were no effects of treatments on plasma metabolites at 28 days (Table 4), supplemental $T_3$ effects on NEFA were noted at 33 days ($P<0.05$; both first period diets) and 36 days ($P<0.05$; control diet during the first period). Supplemental $T_3$ decreased ($P<0.05$) plasma uric acid at all time periods of the second period and, again, regardless of first period dietary treatment. There were inconsistent $T_3$ effects on plasma triglycerides during the second period. For example, supplemental $T_3$

Table 3
Effects initiating methimazole (MMI) treatment at 14 days on responses of broilers given supplemental $T_3$ on in vitro metabolism[1] (Experiment 2)

| 28 day diets | Age | 28–36 day diets | IVL[2] | ME[3] | ICD[3] | AAT[3] |
|---|---|---|---|---|---|---|
| Control | 28 days | | $24.5\pm2.4^a$ | $8.9\pm1.8^b$ | $27.3\pm1.0^a$ | $51.7\pm2.3^a$ |
| MMI | 28 days | | $19.1\pm3.4^a$ | $4.9\pm1.5^a$ | $25.3\pm1.5^a$ | $54.2\pm3.4^a$ |
| | | | | | | |
| Control | 30 days | Control | $25.4\pm3.9^b$ | $13.4\pm0.6^c$ | $27.1\pm3.4^a$ | $55.7\pm6.5^a$ |
| Control | 30 days | $+T_3$ | $15.2\pm1.0^a$ | $9.6\pm0.8^b$ | $28.2\pm1.6^a$ | $56.6\pm3.7^a$ |
| MMI | 30 days | Control | $25.4\pm2.8^b$ | $6.1\pm0.9^a$ | $28.3\pm1.5^a$ | $58.3\pm2.4^a$ |
| MMI | 30 days | $+T_3$ | $35.1\pm1.9^c$ | $10.6\pm1.7^{bc}$ | $33.3\pm3.2^a$ | $70.0\pm4.3^b$ |
| | | | | | | |
| Control | 33 days | Control | $24.4\pm1.8^b$ | $14.3\pm0.3^b$ | $31.8\pm1.6^a$ | $56.8\pm2.9^a$ |
| Control | 33 days | $+T_3$ | $8.0\pm0.7^a$ | $8.5\pm1.0^a$ | $30.6\pm2.6^a$ | $60.0\pm6.8^a$ |
| MMI | 33 days | Control | $28.3\pm2.7^b$ | $10.3\pm1.2^a$ | $25.8\pm1.4^a$ | $61.4\pm3.5^a$ |
| MMI | 33 days | $+T_3$ | $11.5\pm1.6^a$ | $9.7\pm2.2^a$ | $26.4\pm3.4^a$ | $54.5\pm7.6^a$ |
| | | | | | | |
| Control | 36 days | Control | $22.9\pm1.6^b$ | $14.2\pm0.5^b$ | $23.6\pm2.6^a$ | $43.8\pm4.2^a$ |
| Control | 36 days | $+T_3$ | $7.7\pm0.8^a$ | $9.7\pm0.6^a$ | $38.5\pm1.8^b$ | $67.3\pm2.5^b$ |
| MMI | 36 days | Control | $25.9\pm3.2^b$ | $13.6\pm1.5^b$ | $32.9\pm2.0^b$ | $66.4\pm3.9^b$ |
| MMI | 36 days | $+T_3$ | $11.9\pm1.8^a$ | $14.3\pm1.7^b$ | $31.7\pm1.9^b$ | $62.1\pm5.0^b$ |

[a,b,c] Values within an age period with common superscripts are similar ($P<0.05$).
[1] Birds were fed diets containing 1 or 0 g of MMI per kg from 14 to 28 days of age. One-half of the birds were then fed a diet containing 1 mg of $T_3$ from 28 to 36 days of age. The other half of the birds were fed an unsupplemented diet.
[2] IVL, in vitro lipogenesis was noted as micromoles of [2-$^{14}$C]sodium acetate incorporated into hepatic lipids per gram of tissue in a 2-h incubation at 37 °C.
[3] ME, malic enzyme; ICD, NADP-isocitrate dehydrogenase; AAT, aspartate aminotransferase. All activities are expressed as micromoles of reduced or oxidized NAD(P) produced per minute under standardized reaction conditions.

Table 4
Effects initiating methimazole (MMI) treatment at 14 days on responses in broilers given supplemental $T_3$ on plasma metabolites[1,2,3] (Experiment 2)

| 28 day diets | Age | 28–36 day diets | NEFA | Glucose | Uric acid | TG |
|---|---|---|---|---|---|---|
| | | | μequiv/L | mg/100 ml | | |
| Control | 28 days | | $157\pm9.7^a$ | $239\pm12.2^a$ | $6.8\pm0.5^a$ | $71\pm7.3^a$ |
| MMI | 28 days | | $150\pm12.4^a$ | $262\pm19.4^a$ | $8.9\pm1.4^a$ | $117\pm14.7^a$ |
| Control | 30 days | Control | $167\pm14.3^a$ | $267\pm2.1^a$ | $8.1\pm0.8^b$ | $140\pm14.7^b$ |
| Control | 30 days | $+T_3$ | $216\pm13.9^a$ | $257\pm3.5^a$ | $5.0\pm0.8^a$ | $64\pm7.4^a$ |
| MMI | 30 days | Control | $194\pm27.3^a$ | $272\pm12.9^a$ | $14.2\pm1.2^c$ | $156\pm15.9^b$ |
| MMI | 30 days | $+T_3$ | $375\pm29.8^b$ | $234\pm5.5^a$ | $4.3\pm0.4^a$ | $75\pm12.7^a$ |
| Control | 33 days | Control | $181\pm21.3^a$ | $267\pm11.8^a$ | $8.3\pm0.5^b$ | $98\pm6.3^b$ |
| Control | 33 days | $+T_3$ | $621\pm155.1^b$ | $222\pm19.0^a$ | $5.1\pm0.5^a$ | $91\pm12.0^b$ |
| MMI | 33 days | Control | $161\pm12.6^a$ | $259\pm11.9^a$ | $7.1\pm0.6^b$ | $90\pm9.7^b$ |
| MMI | 33 days | $+T_3$ | $502\pm97.9^b$ | $235\pm9.0^a$ | $3.8\pm0.3^a$ | $66\pm4.1^a$ |
| Control | 36 days | Control | $193\pm10.9^a$ | $241\pm5.9^a$ | $7.0\pm0.4^b$ | $112\pm12.1^b$ |
| Control | 36 days | $+T_3$ | $684\pm138.8^b$ | $222\pm15.0^a$ | $4.5\pm0.7^a$ | $76\pm5.1^a$ |
| MMI | 36 days | Control | $204\pm18.6^a$ | $256\pm13.1^a$ | $7.6\pm0.7^b$ | $118\pm12.0^b$ |
| MMI | 36 days | $+T_3$ | $259\pm43.4^a$ | $253\pm4.9^a$ | $3.5\pm0.5^a$ | $65\pm3.4^a$ |

[a,b,c] Values within an age period with common superscripts are similar ($P<0.05$).
[1] Birds were fed diets containing 1 or 0 g of MMI per kilogram from 14 to 28 days of age. One-half of the birds were then fed a diet containing 1 mg of $T_3$ from 28 to 36 days of age.
[2] NEFA, non esterified plasma free fatty acids.
[3] TG, triglycerides.

decreased this variable on days 30 and 36, regardless of the treatments during the first period.

Table 5 presents the carry over effects of $T_3$ supplementation (28–36 days) on metabolic and growth measurements at 49 days. It is important to understand that all birds had been fed the same control diet from day 36 to day 49. There were no significant differences in body weight among the four groups. Both plasma $T_3$ and $T_4$ were lowest ($P<0.05$) in birds fed the control diet during the first period and the $T_3$-supplemented diet during the second period. This treatment effect was also

noted for both IVL and ME. In contrast, plasma uric acid was lowest ($P<0.05$) in birds fed MMI during the first period and supplemental $T_3$ during the second period.

Tables 6 and 7 summarize dose–response relationships between MMI and metabolic parameters. There was a significant (Table 6; $P<0.01$) inverse linear relationship between 28-day body weight and the MMI dose. Both of the two higher MMI concentrations (0.5 and 1.0 g MMI/kg diet) significantly reduced plasma lactate compared to the lowest concentration of MMI and the control diets (0.25 and 0 g

Table 5
Effects initiating methimazole (MMI) treatment at 14 days and $T_3$ replenishment on responses in broilers at 49 days of age[1] (Experiment 2)

| | Diets from 14 to 28 days | | | |
|---|---|---|---|---|
| | Control | | MMI | |
| | Diets from 28 to 36 days | | | |
| | Control | $+T_3$ | Control | $+T_3$ |
| Body weight (g) | $2481\pm100.4^a$ | $2162\pm155.1^a$ | $2493\pm102.5^a$ | $2204\pm32.4^a$ |
| Plasma $T_3$ (ng/ml) | $2.1\pm0.1^b$ | $1.4\pm0.2^a$ | $2.1\pm0.1^b$ | $2.3\pm0.1^b$ |
| Plasma $T_4$ (ng/ml) | $19.2\pm0.8^b$ | $11.0\pm2.1^a$ | $16.2\pm0.8^b$ | $20.4\pm2.7^b$ |
| Plasma NEFA (μequiv/L) | $161\pm11.9^a$ | $253\pm43.3^b$ | $252\pm37.6^b$ | $312\pm129.2^b$ |
| Plasma glucose (mg/100 ml) | $257\pm7.8^a$ | $223\pm20.0^a$ | $215\pm2.6^a$ | $219\pm9.9^a$ |
| Plasma uric acid (mg/100 ml) | $7.6\pm0.6^c$ | $7.7\pm0.5^c$ | $6.0\pm0.5^b$ | $4.7\pm0.2^a$ |
| Plasma triglycerides (mg/100 ml) | $111\pm8.8^b$ | $77\pm22.5^a$ | $74\pm11.1^a$ | $76\pm6.8^a$ |
| IVL[2] | $20.8\pm0.5^b$ | $9.7\pm1.5^a$ | $15.8\pm2.7^b$ | $17.7\pm2.1^c$ |
| ME[3] | $12.0\pm0.9^b$ | $6.6\pm2.0^a$ | $14.3\pm1.3^b$ | $11.0\pm1.4^b$ |
| ICD[3] | $29.4\pm1.3^a$ | $24.7\pm3.6^a$ | $51.8\pm11.9^b$ | $33.8\pm1.7^a$ |
| AAT[3] | $55.6\pm2.9^b$ | $41.3\pm4.9^a$ | $43.1\pm3.8^a$ | $48.5\pm3.1^a$ |

[a,b,c] Values within a row with common superscripts are similar ($P<0.05$).
[1] Birds were fed diets containing 1 or 0 g of MMI per kilogram from 14 to 28 days of age. One-half of the birds were then fed a diet containing 1 mg of $T_3$ from 28 to 36 days of age. The birds were then fed a common diet from 36 to 49 days of age.
[2] IVL, in vitro lipogenesis was noted as micromoles of $[2-^{14}C]$sodium acetate incorporated into hepatic lipids per gram of tissue in a 2-h incubation at 37 °C.
[3] ME, malic enzyme; ICD, NADP-isocitrate dehydrogenase; AAT, aspartate aminotransferase. All activities are expressed as μmoles of reduced or oxidized NAD(P) produced per minute under standardized reaction conditions.

Table 6
Effects of graded doses of methimazole (MMI) given to birds growing from 7 to 28 days of age on growth and plasma metabolites[1] (Experiment 3)

| MMI | Weight | NEFA[2] | Glucose | Lactate | TG[3] | $T_3$ | $T_4$ |
|---|---|---|---|---|---|---|---|
| | | µequiv/L | mg/100 ml | | | ng/ml | |
| 0 | $1244 \pm 27.1^d$ | $156 \pm 8.8^a$ | $256 \pm 34.6^a$ | $41 \pm 2.7^b$ | $71 \pm 3.8^a$ | $1.5 \pm 0.1^c$ | $7.5 \pm 0.6^c$ |
| 0.25 | $957 \pm 34.8^c$ | $188 \pm 11.5^a$ | $251 \pm 32.9^a$ | $40 \pm 4.1^b$ | $74 \pm 4.6^a$ | $1.7 \pm 0.2^c$ | $4.9 \pm 0.6^b$ |
| 0.5 | $840 \pm 46.1^b$ | $183 \pm 16.0^a$ | $252 \pm 38.9^a$ | $22 \pm 1.7^a$ | $76 \pm 4.8^a$ | $0.7 \pm 0.2^b$ | $2.1 \pm 0.4^a$ |
| 1.0 | $704 \pm 24.1^a$ | $184 \pm 26.2^a$ | $296 \pm 32.6^a$ | $23 \pm 2.4^a$ | $72 \pm 7.9^a$ | $0.2 \pm 0.1^a$ | $1.6 \pm 0.5^a$ |

[a,b,c,d] Values within a column period with common superscripts are similar ( $P < 0.05$).
[1] Birds were fed diets containing 0, 0.25, 0.50 and 1.0 g MMI per kilogram diet, starting at 7 days of age and continuing until 28 days of age.
[2] NEFA, non esterified plasma free fatty acids.
[3] TG, triglycerides.

M < MI/kg diet). Feeding the greatest level of MMI (1.0 g MMI/kg diet) resulted in the lowest concentrations of plasma $T_3$ and $T_4$ and feeding the control diet the highest concentrations of these hormones. Feeding the next greatest level of dietary MMI resulted in a greater plasma $T_3$ concentration that was still less than that attained by feeding either the lowest level of dietary MMI (0.25 g/kg diet) or the control diet.

Both ME and ICD were significantly lower (Table 7; $P < 0.01$) in birds fed the highest concentration of dietary MMI (1.0 g MMI/kg diet) compared to the other three treatment concentrations. Although, as stated, the next greatest concentration of dietary MMI (0.5 g MMI/kg diet) resulted in significantly greater values for both IVL and ME when compared to the values attained by feeding the greatest concentration of MML, these values were still less than those for the lowest concentration of MMI (0.25 g MMI/kg diet) and for the control diet. In contrast, neither ICD nor AAT were affected by MMI.

## 4. Discussion

We have demonstrated two methods of altering plasma thyroid hormones by altering MMI regimens (1) changing the MMI dosing interval while maintaining a constant dose and (2) changing the MMI dose while maintaining the same dosing interval. It was noted that initiating MMI at 14 days (1 g/kg diet) depressed circulating $T_3$ and $T_4$ but affected neither body

Table 7
Effects of graded doses of methimazole (MMI) given to birds growing from 7 to 28 days of age on in vitro metabolism[1] (Experiment 3)

| MMI | IVL[2] | ME[3] | ICD[3] | AAT[3] |
|---|---|---|---|---|
| 0 | $36.5 \pm 1.4^c$ | $28.2 \pm 2.1^c$ | $39.6 \pm 1.6^a$ | $47.8 \pm 2.1^a$ |
| 0.25 | $38.5 \pm 2.4^c$ | $28.1 \pm 2.6^c$ | $36.7 \pm 1.9^a$ | $40.7 \pm 1.7^a$ |
| 0.5 | $20.8 \pm 2.1^b$ | $9.2 \pm 1.9^b$ | $39.1 \pm 2.9^a$ | $46.4 \pm 2.3^a$ |
| 1 | $14.2 \pm 1.0^a$ | $3.4 \pm 0.6^a$ | $40.1 \pm 1.1^a$ | $47.7 \pm 1.7^a$ |

[a,b,c,d] Values within a column age period with common superscripts are similar ( $P < 0.05$).
[1] Birds were fed diets containing 0, 0.25, 0.50 and 1.0 g MMI per kilogram diet, starting at 7 days of age and continuing until 28 days of age.
[2] IVL, in vitro lipogenesis was noted as micromoles of [2-$^{14}$C]sodium acetate incorporated into hepatic lipids per gram of tissue in a 2-h incubation at 37 °C.
[3] ME, malic enzyme; ICD, NADP-isocitrate dehydrogenase; AAT, aspartate aminotransferase. All activities are expressed as micromoles of reduced or oxidized NAD(P) produced per minute under standardized reaction conditions.

weight nor lipogenesis at 28 days. In contrast, while MMI doses (0, 0.25, 0.5 and 1.0 g/kg diet) resulted in significant dose–response depressions in body weight, the diet containing 0.5 g MMI/kg was the first diet to significantly depress lipogenesis. Thus, it did not appear possible to alter thyroid metabolism sufficiently to change lipogenesis without adversely affecting body weight.

The data from this study adds to our previous findings showing that restoration of normal levels of $T_3$ is necessary for optimum lipid metabolism in the chicken (Rosebrough and McMurtry, 2003). The present study shows that supplemental $T_3$ enhanced lipogenesis in the hypothyroid chicken, but that enhancement only lasted until restoration of normal levels of plasma $T_3$ occurred. At this point, it should be noted that $T_3$ supplementation did not restore lipogenesis to control levels although plasma $T_3$ concentrations were much greater than controls. Nonetheless, restoration of plasma $T_3$ is important because $T_3$ regulates both the metabolism of chickens and the flux of calories supporting metabolism. In addition, $T_3$ may alter the sensitivity of an organ to other regulatory hormones and tissue factors as well as directly influence the metabolic rate of that organ (McNabb et al., 1991).

Although the data in the present study indicate that $T_3$ depresses lipogenesis in the euthyroid bird, it has little effect on the activities of certain lipogenic enzymes. In this respect, our data provide a contrast to other work. For example, Clarke and Hembree (1990) found an increase in malic enzyme activity in rats injected with $T_3$. Previous articles describing metabolism in rodents work also showed that $T_3$ increased lipogenesis (Roncari and Murthy, 1975) and lipogenic enzyme activities (Mariash and Oppemheimer, 1984). Triiodothyronine administration has been reported to increase rat liver malic enzyme mRNA abundance by possibly altering transcriptional events, nuclear processing or mRNA turnover (Magnuson and Nikodem, 1983). Work by Jump et al. (1988) indicated that $T_3$ stimulates transcription by forming a nuclear hormone–receptor complex that enhances transcription. Lastly, it should follow that a relative increase in mRNA abundance increases enzyme protein synthesis and activity (Clarke and Hembree, 1990).

In summary, the findings of this study continue to support a relationship between thyroid status and fat metabolism and may help to explain some of the conflicts concerning the role of the thyroid in lipid metabolism. Although our previous

findings show that exogenous $T_3$ depresses de novo lipogenesis (Rosebrough et al., 1992), it can also be shown that normal levels of endogenous $T_3$ are required for this effect (Song et al., 1988, 1989; Rosebrough and McMurtry, 2003). In many cases, MMI can be given to birds to alter circulating thyroid hormone levels without affecting body weight at 28 days of age. One regimen (1 g MMI/kg diet given at 14 days of age) did not depress in vitro lipogenesis at 28 days of age. In most cases, supplemental $T_3$ will restore lipogenic potential in hypothyroid birds and depress that same process in euthyroid birds. It is also evident from these studies that the absolute dose of dietary MMI may be less important than the actual period of exposure to MMI.

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